Journal of Chromatography A, 1218 (2011) 1393–1398

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Characterization of new *R*-naphthylethyl cyclofructan 6 chiral stationary phase and its comparison with *R*-naphthylethyl β-cyclodextrin-based column

Květa Kalíková[a], Lucie Janečková[b], Daniel W. Armstrong[c], Eva Tesařová[a,*]

[a] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Albertov 2030, 128 43 Prague 2, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Science, Charles University in Prague, Albertov 2030, 128 43 Prague 2, Czech Republic
[c] Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, TX 76019, USA

ARTICLE INFO

Article history:
Received 26 November 2010
Received in revised form 4 January 2011
Accepted 11 January 2011
Available online 15 January 2011

Keywords:
Chiral separation
R-naphthylethyl cyclofructan 6
R-naphthylethyl β-cyclodextrin
LFER
Binaphthyl catalysts

ABSTRACT

Derivatized cyclofructans have been recently introduced as a new class of chiral selectors with great application potential. In this study, a *R*-naphthylethyl-functionalized cyclofructan 6 based chiral stationary phase (RN CF6 CSP) was used for separation of substituted binaphthyl catalysts in the normal phase HPLC mode. Dominant interaction types that play a role in the separation mechanism were revealed by a linear free energy relationship (LFER) method. In order to evaluate the contribution of the substituent on the cyclofructan structure to retention, the *R*-naphthylethyl-functionalized β-cyclodextrin (RN CD) CSP was chosen for comparison. Retention factors of 46 widely different solutes, with known solvation parameters, were determined on each of the columns under the same mobile phase compositions used for the enantiomeric separations. The LFER results showed that hydrogen bond acidity and polarity/polarizibility have the greatest impact on retention and enantioresolution on the RN CF6 CSP. The equal influence of the naphthylethyl substituent on the both CSPs was also confirmed while the effects of the basic cyclofructan *versus* cyclodextrin structures were different. The addition of trifluoroacetic acid to the hexane/propane-2-ol mobile phase was negligible on the RN CF6 CSP for the majority of atropoisomers except for one with ionizable functional groups. The RN CF6 column was shown to be more suitable for enantioseparation of the binaphthyl catalysts than the RN CD column. Higher retention offered by the latter CSP had no positive effect on the enantioresolution.

1. Introduction

Enantiomeric separations have been the focus of considerable attention for nearly three decades due to their importance especially in areas of pharmaceutical, agrochemical and food science. HPLC with chiral stationary phases (CSPs) is far and away the most powerful and widely used technique for enantioselective separations at both the analytical and preparative scales. A variety of CSPs usually bonded or adsorbed to silica gel have been reported [1]. A new class of chiral selectors based on cyclofructan was introduced in 2009 and shown to have potential both for HPLC [2–4] and CZE [5]. Cyclofructans (CFs) refer to a group of macrocyclic oligosaccharides that consist of six or more β-(2→1) linked D-fructofuranose units [6,7]. Each fructofuranose unit contains four stereogenic centers and three hydroxyl groups. Native CFs have rather limited enantioselectivity in HPLC [2]. The CF hydroxyl groups can be derivatized with aliphatic or aromatic groups. These functionalized forms of CFs show improved and unique separation abilities over a wide range of analytes. Derivatization of native chiral molecules with aromatic moieties is a common strategy used to enhance their chiral recognition abilities [4]. The recently introduced RN CF6 column utilizes *R*-naphthylethyl-functionalized cyclofructan 6 (CF6, contains six fructofuranose units) as the chiral selector. This new CSP shows very good enantioselectivity toward a variety of enantiomers except for primary amines. As the chiral selector is covalently bonded to the silica gel support, this CSP is compatible with all common organic solvents. In principle it can be operated in all three modes – normal, reversed phase and polar organic. However, better resolution was achieved in the normal phase mode, due to higher selectivity, which also offers the potential for preparative separations [2].

Binaphthyl derivatives have been extensively used to control asymmetric processes. Their outstanding chiral discrimination abilities are derived from their rigidity and spatial arrangement [8,9]. The chirality of these compounds is caused by restricted rotation around the single bond in the binaphthyl skeleton [10,11]. Although the basic structure of the binaphthyl derivatives is similar, the substituents and their position significantly affect their properties. For more information about these compounds see Refs. [9,12–14].

* Corresponding author. Tel.: +420 2 21951296; fax: +420 2 24919752.
E-mail address: tesarove@natur.cuni.cz (E. Tesařová).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.023

One of the comprehensive methods that allow characterization of stationary phase/separation systems and allows a better understanding of the relevant intermolecular interactions, which play a role in the separation processes, is the linear free energy relationship (LFER) [15]. The LFER can independently describe the contributions of individual interactions to the retention. The overall applicability of the LFER model has been presented in numerous reports in recent years (e.g. [16–21]).

One of the more widely accepted representations of the LFER was proposed by Abraham et al. [22] and now it is used in the following form:

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

where k is the solute retention factor. The independent variables in Eq. (1) are solute descriptors and denote specific solute properties: E is the solute excess molar refraction modeling the solute polarizability due to n- and/or π-electron pairs, S is the solute dipolarity/polarizibility parameter, A is the effective or overall hydrogen bond acidity, B is the effective or overall hydrogen bond basicity and V is the McGowan's characteristic molecular volume calculated from the solute structure [23–26]. The descriptors characterize properties of the solute molecules and account for the differences among them. A representative series of analytes must be selected to evaluate the chromatographic system. These compounds should be structurally diverse and the distribution of the individual descriptors should equally cover the whole range of interactions [27,28]. The coefficients in Eq. (1) are determined by multivariate regression analysis and reflect the individual types of molecular interactions acting in the given separation system. Since in HPLC Eq. (1) is applied to the distribution between two phases, the regression coefficients refer to differences between the phases, i.e., a given stationary phase and a fixed composition of the mobile phase. The c constant is the intercept obtained in the regression calculation; it depends on the separation system used but it does not reflect any interaction [29]. The value e reflects the difference in propensity of the stationary and the mobile phases to interact with solute n- and π-electron pairs; s reflects difference in dipolarity/polarizability between the phases; a refers to the difference in hydrogen bond basicity between the stationary and the mobile phases; b is equal to the difference in hydrogen bond donating properties and v reflects the difference in hydrophobicity between the stationary and the mobile phases.

As the LFER model characterizes the chromatographic system as a whole, comparisons of different stationary phases must be done at the same mobile phase composition. However, the mobile phase is an important factor affecting separation, therefore the characterization of a HPLC separation system should be performed under various mobile phase compositions. Complete or optimal model parameters can be obtained from the multivariate regression analysis. The complete model involves all the regression coefficients while the optimal model utilizes just the statistically significant values. Ordinary regression coefficients serve well for comparison of different stationary phases at the same mobile phase composition. Statistically derived standardized coefficients equilibrate influences of the different units, their mean values are zero, and the standard deviations (SDs) are the same for all of them. Therefore, the standardized coefficients are well-suited to analyze the various interactions within one separation system, composed of a given stationary phase and a mobile phase [30].

This work is focused on a study of separation properties of the new cyclofructan-based chiral stationary phase – *R*-naphthylethyl-functionalized cyclofructan 6 (RN CF6) CSP, and comparison of its separation abilities with *R*-naphthylethyl carbamoyl β-cyclodextrin (RN CD) CSP, using LFER method. These CSPs have the same substituent, *R*-naphthylethyl carbamate group, and isomeric saccharide units, six fructofuranose and seven glucopyranose units in CF6 and β-CD, respectively [31,32]. The paper is aimed at elucidating the molecular interaction mechanisms, i.e., revealing the types of interactions responsible for retention. The application of LFER to enantiomeric separations is not explicit as no chiral term is involved in the equation but the calculated regression coefficients can serve as a tool for estimation of the interactions "useful" for chiral discrimination. Separation performance of the new RN CF6 CSP is demonstrated on binaphthyl catalysts. Their structure seems to be well-suited for interaction with the naphthylethyl substituent of the chiral selector.

2. Experimental

2.1. Instrumentation

All chromatographic measurements were performed on Waters Alliance system (Waters Chromatography, Milford, MA, USA) consisting of a Waters 2695 Separation Module, a Waters 2996 Photodiode Array Detector, a Waters 717 plus Autosampler, and a Waters Alliance Series column heater. Empower software was used for process control and data handling. Chromatographic columns RN CF6 (*R*-naphthylethyl carbamate cyclofructan 6 CSP bonded to silica gel) and Cyclobond I 2000 RN (RN CD; *R*-naphthylethyl carbamate β-cyclodextrin CSP bonded to silica gel) were used in this work. The dimensions of both columns were 250 mm × 4.6 mm i.d.; particle size 5 μm. RN CF6 column has been prepared at the Department of Chemistry and Biochemistry, University of Texas at Arlington (Arlington, TX, USA). Cyclobond I 2000 RN column is a product of ASTEC (Whippany, NJ, USA). The columns and samples were thermostated at 25 °C. Detection was performed at 254 nm. The flow rate was 1 mL/min for all measurements.

2.2. Chemicals

Organic solvents of HPLC grade, n-hexane (hex), propane-2-ol (isopropanol, IPA) and methanol were products of Sigma–Aldrich (Steinheim, Germany). Trifluoroacetic acid (TFA; 99.8% purity) was purchased from Merck (Darmstadt, Germany). The solutes for LFER were of analytical grade purity and were purchased from Sigma–Aldrich (St. Louis, MO, USA). They were selected to cover a wide range of chemical properties. The list of the 46 solutes used and their corresponding solvation parameters are summarized in Table 1. The chiral compounds (binaphthyl catalysts) have been synthesized as racemates at the Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague (Prague, Czech Republic) [8,9]. The structures of binaphthyl catalysts are shown in Fig. 1.

Stock solutions of solid test compounds were prepared in concentration of 1 mg/mL and stock solutions of liquid samples were diluted to obtain 20 μL/mL using methanol as a solvent.

Mobile phases were composed of hexane and propane-2-ol in various ratios and/or hexane and propane-2-ol mixtures with the small additions of trifluoroacetic acid.

2.3. Procedures

The retention times of the test solutes were measured in triplicates in all the chromatographic systems studied. The void volumes were determined using system peaks obtained by injection of n-hexane to individual separation systems. The retention times were calculated from the peak maxima. As the detector responses of the test analytes were kept rather low, the effect of peak shape was not critical in the evaluation of retention times used for the calculations. The average SD of sequential measurements of the retention factor did not exceed 1.5%. The regression coefficients of the LFER equation were obtained from a series of measurements of the retention

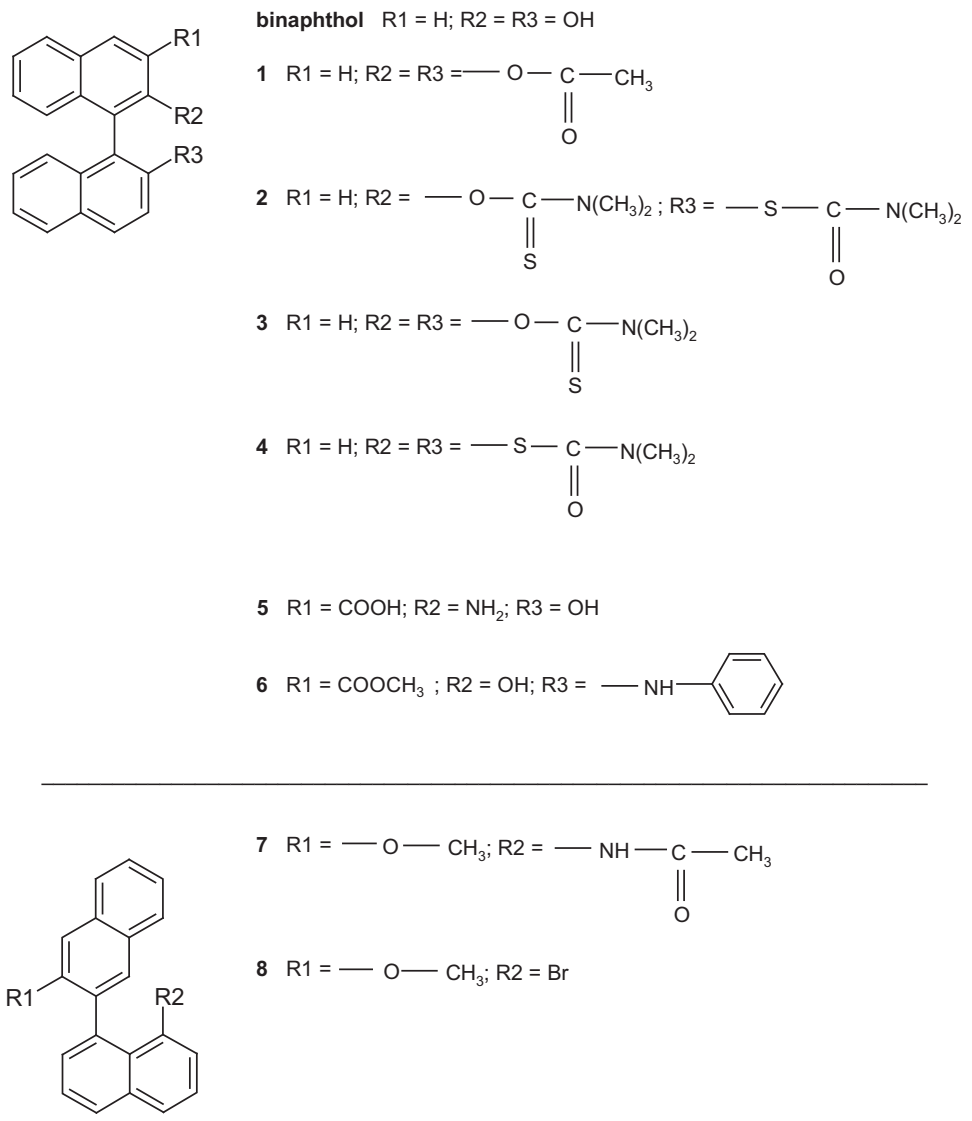

Fig. 1. Structures of the binaphthyl derivatives.

data of the set of 46 structurally different test solutes with known solvation parameters [23,28,33] that are shown in Table 1. The coefficient values were calculated for each separation system, i.e., CSP and mobile phase composition, by multiple linear regression analysis of log *k* against the solute descriptors using NCSS software (NCSS, Kaysville, UT, USA) [34]. The results were determined for both the complete model utilizing all regression coefficients and the optimal model handling just the statistically significant regression parameter values.

3. Results and discussion

3.1. Enantiomeric separation of binaphthyl catalysts

The cyclofructan-based RN CF6 column, a representative of this novel class of CSPs, was chosen for enantioseparation of substituted binaphthyl catalysts in the normal phase separation mode. The column was selected because the structure of the chiral selector, the cyclofructan derivative (*R*-naphthylethyl group), seemed to be compatible with the structure of the binaphthyl derivatives. Mobile phases were composed of hexane and propane-2-ol in various ratios and also the addition of trifluoroacetic acid into these mobile phases was tested. The influence of the addition of the acid to the hex/IPA 80/20 (*v*/*v*) mobile phase is obvious from the results summarized in Table 2. The presence of TFA in the mobile phase did not have significant effect on the retention and separation of the majority of the analytes and their atropoisomers. The acidified mobile phase just slightly reduced the retention values.

One exception to this was analyte 5 which exhibited an exceptional behavior (Fig. 2). This binaphthyl derivative has accessible ionizable groups, and so the addition of TFA significantly improved its enantioresolution (the *R*s increased from 0.14 to 1.43 without and with the acid, respectively, even though retention was reduced in the latter case). In addition, baseline resolution was achieved for atropoisomers of derivative 3 and partial separation was obtained for atropoisomers of binaphthol and solutes 1 and 2 (see Table 2) under both mobile phase compositions. Atropoisomers of analytes 4, 6, 7 and 8 were not separated in any chromatographic system tested. A comparison of the retention and separation of atropoisomers of compounds 2, 3 and 4 is interesting given the similarity of their substituents (see Fig. 1). Analytes 3 and 4 have their oxygen and sulfur moieties reversed. Analyte 2 has one substituent the same as analyte 3 and one substituent the same as analyte 4. Analyte 3 has the lowest retention factor and the highest enantioresolution.

Analyte 4 had the greatest retention among all these analytes but its atropoisomers were not separated. The retention factor of

Table 1
Set of test analytes and their solvation parameters.

Analyte	*E*	*S*	*A*	*B*	*V*
Benzamide	0.99	1.50	0.49	0.67	0.973
2-Naphthol	1.52	1.08	0.61	0.40	1.144
Resorcinol	0.98	1.00	1.10	0.58	0.834
Benzophenone	1.45	1.50	0.00	0.50	1.481
Hydroquinone	1.00	1.00	1.16	0.60	0.834
1,2-Cresol	0.84	0.86	0.52	0.31	0.916
Benzonitrile	0.74	1.11	0.00	0.33	0.871
1,3-Cresol	0.82	0.88	0.57	0.34	0.916
Benzylalcohol	0.80	0.87	0.33	0.56	0.916
Benzene	0.61	0.52	0.00	0.14	0.716
Naphthalene	1.34	0.92	0.00	0.20	1.085
Pyrocatechol	0.97	1.07	0.85	0.52	0.834
Dibenzothiophene	1.96	1.31	0.00	0.18	1.379
Ethylbenzene	0.61	0.51	0.00	0.15	0.998
Benzaldehyde	0.82	1.00	0.00	0.39	0.873
Toluene	0.60	0.52	0.00	0.14	0.857
1,2-Toluidine	0.97	0.92	0.23	0.45	0.957
Biphenyl	1.36	0.99	0.00	0.22	1.324
Phenanthrene	2.06	1.29	0.00	0.26	1.454
1,2,3-Trichlorobenzene	1.03	0.86	0.00	0.00	1.084
3-Nitrotoluene	0.87	1.10	0.00	0.25	1.032
1,2-Xylene	0.66	0.56	0.00	0.16	0.998
Bromobenzene	0.88	0.73	0.00	0.09	0.891
2-Nitrotoluene	0.87	1.11	0.00	0.27	1.032
1,3-Xylene	0.62	0.52	0.00	0.16	0.998
Chlorobenzene	0.72	0.65	0.00	0.07	0.839
1,4-Xylene	0.61	0.52	0.00	0.16	0.998
2-Chlorophenol	0.85	0.88	0.32	0.31	0.898
3-Chlorophenol	0.91	1.06	0.69	0.15	0.898
4-Chlorophenol	0.92	1.08	0.67	0.21	0.898
2-Nitrophenol	1.02	1.05	0.05	0.37	0.949
4-Nitrophenol	1.07	1.72	0.82	0.26	0.949
3-Hydroxybenzaldehyde	0.99	1.38	0.74	0.40	0.932
Acetone	0.18	0.70	0.04	0.49	0.547
Aniline	0.96	0.96	0.26	0.41	0.816
Anthracene	2.29	1.34	0.00	0.26	1.454
Tetrachlorobenzene	1.18	0.92	0.00	0.00	1.206
Pyrene	2.81	1.71	0.00	0.29	1.585
Caffeine	1.50	1.60	0.00	1.33	1.364
1,4-toluidine	0.92	0.95	0.23	0.45	0.957
Pyridine	0.63	0.84	0.00	0.52	0.675
Theophylline	1.50	1.60	0.54	1.34	1.222
Thymine	0.80	1.00	0.44	1.83	0.893
Ethylacetate	0.11	0.62	0.00	0.45	0.747
Uracil	0.81	1.00	0.44	1.00	0.752
Phenol	0.81	0.89	0.60	0.30	0.775

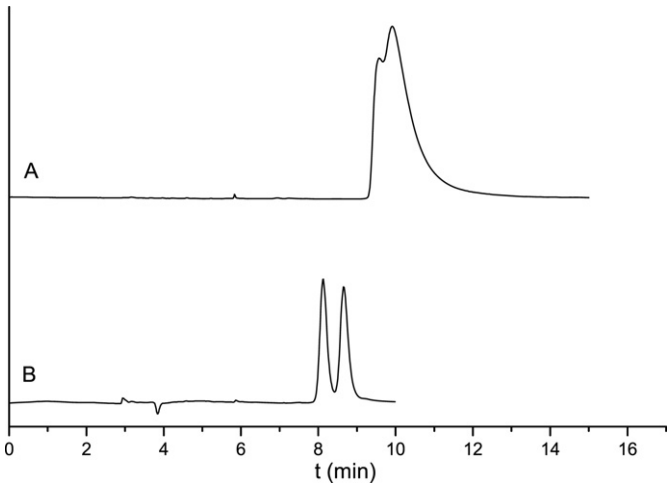

Fig. 2. Chiral separation of analyte 5 atropoisomers on the RN CF6 column. Mobile phase compositions: A: n-hexane/IPA 80/20 (v/v); B: n-hexane/IPA/TFA 80/20/0.5 (v/v/v); column and sample temperatures: 25 °C; flow rate: 1 mL/min; UV detection: 254 nm.

analyte 2 is between those of analytes 3 and 4, and the resolution of its atropoisomers is higher than that of analyte 4 ($R = 0$) and lower than that of analyte 3. It can be concluded that the substituent type of analyte 3 has a positive effect on the chiral discrimination process whereas the substituent of analyte 4 has the opposite effect.

β-Cyclodextrin based RN CD column also was tested for the separation of these compounds with mobile phases of the same compositions as those used with the cyclofructan-based CSP (Table 2). The RN CD CSP was selected because the β-cyclodextrin derivative contains the same substituent (naphthylethyl carbamoyl group) on an oligosaccharide base as the RN CF6 chiral selector. A strong effect of the addition of TFA to the mobile phase on retention of the binaphthyl derivatives was observed on the RN CD column. The retention was substantially reduced with the acidified mobile phase. Unfortunately, no significant enantioseparation of the atropoisomers was observed on this CSP in the normal separation mode. Only atropisomers of compound 5 were partly resolved in the mobile phase with TFA (Fig. 3).

A comparison of the results obtained on these two chiral stationary phases shows that the RN CF6 column is more suitable for enantioseparation of the binaphthyl catalysts. The retention of

Table 2
The chromatographic parameters of the chiral analytes using CF6 RN and CD RN columns; k_1, retention factor of the first eluted atropoisomer; α, selectivity; R, resolution.

Mobile phase	Analyte	CF6 RN			CD RN		
		k_1	α	R	k_1	α	R
hex/IPA/TFA 80/20/0.0 (v/v/v)	Binaphthol	1.38	1.08	1.08	7.67	1.00	0.00
	1	0.94	1.10	1.32	1.56	1.00	0.00
	2	2.17	1.07	0.98	4.02	1.00	0.00
	3	1.35	1.16	1.95	3.09	1.00	0.00
	4	6.34	1.00	0.00	8.26	1.00	0.00
	5	2.17	1.05	0.14	8.55	1.00	0.00
	6	3.19	1.00	0.00	5.52	1.00	0.00
	7	0.89	1.00	0.00	2.17	1.00	0.00
	8	0.43	1.00	0.00	0.85	1.00	0.00
hex/IPA/TFA 80/20/0.5 (v/v/v)	Binaphthol	1.47	1.08	1.04	3.11	1.00	0.00[a]
	1	0.89	1.10	1.44	1.26	1.00	0.00
	2	2.02	1.07	0.95	3.04	1.00	0.00
	3	1.29	1.17	1.87	2.42	1.00	0.00
	4	5.38	1.00	0.00	5.83	1.00	0.00
	5	1.76	1.10	1.43	3.44	1.05	0.31
	6	2.76	1.00	0.00	4.23	1.00	0.00
	7	0.88	1.00	0.00	1.61	1.00	0.00
	8	0.44	1.00	0.00	0.56	1.00	0.00

[a] Slight indication of enantioseparation.

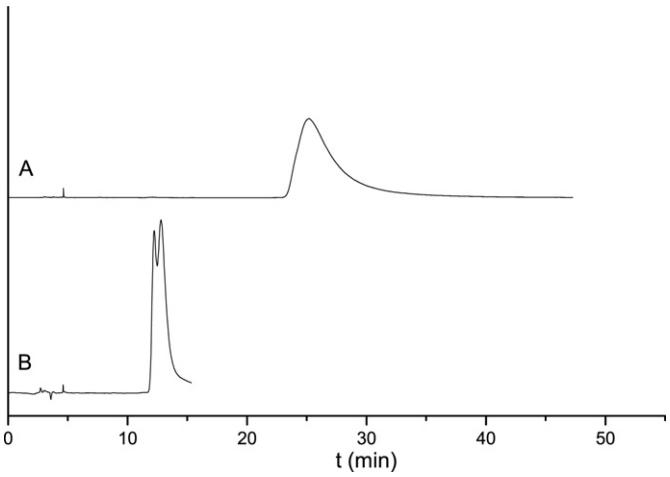

Fig. 3. Chiral separation of analyte 5 atropoisomers on the RN CD column. Mobile phase compositions: A: n-hexane/IPA 80/20 (v/v); B: n-hexane/IPA/TFA 80/20/0.5 (v/v/v). For other experimental conditions see caption to Fig. 2.

all the tested analytes was much higher if the RN CD column was used. Nevertheless, the higher retention had no positive impact on enantioresolution.

3.2. Comparison of RN CF6 and RN CD columns using LFER model

The LFER model was used in order to better understand the interactions involved in the retention and separation of the substituted binaphthyl derivatives on the two chiral stationary phases. This approach can reveal contributions of the individual interaction types, obtained from regression coefficients v, a, b, s and e of Eq. (1).

The LFER data for the separation systems previously discussed in Section 3.1 are summarized in Table 3. The table shows the regression coefficients obtained from the complete and the optimal models of LFER and also the standardized coefficients of the optimal model. Correlation of the LFER data with experimental results (plot of the experimental $\log k$ against calculated $\log k$) achieved for the set of 46 structurally diverse test solutes on the both CSPs did not show any serious outliers, correlation coefficients of linear regression fits were always higher than 0.93. Lower p-values of the optimal model than those of the complete model (see Table 3) show that the regression coefficients of the former model are more significant [34]. Due to the fact that insignificant interactions are also included in the complete LFER model, the optimal model offers a better tool for comparison of the chromatographic systems studied in this work.

Negative values of the regression coefficient v (representing difference in hydrophobicity between the stationary and the mobile phases) obtained for all four separation systems show that hydrophobic interactions are preferred in the mobile phase. This is legitimate in a normal separation mode where the hydrophobicity of a mobile phase is higher than that of a stationary phase. The v values show a clearly defined trend, i.e., the absolute v value decreases if TFA is added to the mobile phase no matter what CSP is used. If we compare the results for the tested columns obtained in the separation systems with the same mobile phase the difference in hydrophobicity between the stationary and the mobile phases is higher for the system employing RN CF6 column. Thus, the RN CF6 stationary phase can be considered more polar than the RN CD column. Obtained results (v values) correspond with the retention of binaphthyl derivatives (Table 2), i.e., their retention factors are higher in chromatographic systems with the RN CD column. This confirms the general idea that cyclofructans do not possess central hydrophobic cavity as do cyclodextrins [35,36]. The regression coefficient a (describing difference in hydrogen bond basicity) is statistically insignificant for RN CF6 column in the both mobile phases tested. So this type of interaction is not involved in the optimal model. That means that the basicity (ability to accept protons) of this stationary phase is low and similar to that of the

Table 3
Regression coefficients of the LFER equation and correlation coefficient R.

Column	Mobile phase	Model	v	a	b	s	e	c	R
RN CF6	hex/IPA/TFA 80/20/0.0 (v/v/v)	C.M.	−1.623	−0.035	1.669	0.995	0.278	−0.659	0.957
		±95% CI	0.704	0.247	0.312	0.364	0.328	0.475	
		p	0.000	0.776	0.000	0.000	0.092	0.008	
		O.M.	−1.169	x	1.596	1.128	x	−0.944	0.953
		±95% CI	0.382		0.302	0.298		0.307	
		p	0.000		0.000	0.000		0.000	
		STD	−0.407	x	0.638	0.572	x	0.000	
	hex/IPA/TFA 80/20/0.5 (v/v/v)	C.M.	−1.019	0.092	1.556	0.759	0.130	−0.823	0.967
		±95% CI	0.623	0.216	0.237	0.299	0.275	0.421	
		p	0.002	0.395	0.000	0.000	0.342	0.000	
		O.M.	−0.918	x	1.535	0.891	x	−0.892	0.965
		±95% CI	0.316		0.231	0.244		0.254	
		p	0.000		0.000	0.000		0.000	
		STD	−0.338	x	0.705	0.482	x	0.000	
RN CD	hex/IPA/TFA 80/20/0.0 (v/v/v)	C.M.	−1.381	0.506	0.928	1.033	0.211	−0.342	0.931
		±95% CI	0.898	0.337	0.331	0.503	0.416	0.647	
		p	0.004	0.004	0.000	0.000	0.309	0.290	
		O.M.	−1.037	0.553	0.901	1.148	x	−0.581	0.929
		±95% CI	0.591	0.324	0.327	0.449		0.443	
		p	0.001	0.001	0.000	0.000		0.012	
		STD	−0.365	0.280	0.434	0.519	x	0.000	
	hex/IPA/TFA 80/20/0.5 (v/v/v)	C.M.	−0.963	0.284	1.455	0.831	0.194	−0.896	0.966
		±95% CI	0.600	0.211	0.255	0.293	0.265	0.414	
		p	0.002	0.010	0.000	0.000	0.147	0.000	
		O.M.	−0.623	0.330	1.421	0.906	x	−1.114	0.964
		±95% CI	0.382	0.204	0.255	0.279		0.291	
		p	0.002	0.002	0.000	0.000		0.000	
		STD	−0.234	0.189	0.590	0.484	x	0.000	

CI represents ±95% confidence interval. x, insignificant interaction; C.M., complete model of the LFER equation; O.M., optimal model of the LFER equation; STD, standardized coefficients of the optimal LFER equation; p, statistical p-value. The p-values express probability of the error that the individual coefficient does not contribute to the model, i.e., p-values represent the significance of the individual coefficients.

mobile phases. On the other hand, the regression coefficients *a* are significant in both the systems with the RN CD column. The *a* values are positive, i.e., this type of interaction contributes to the retention. Lower value of the coefficient *a* was observed in the system with TFA in the mobile phase. TFA can occupy some of the proton accepting sites on the stationary phase and in this way reduce their availability to the analytes. The regression coefficients *b* (expressing hydrogen bond acidity difference) are positive in all chromatographic systems studied. The hydrogen bond acidity of the RN CF6 and RN CD CSPs is always higher than that of the both mobile phases used. Moreover, RN CF6 CSP has higher hydrogen bond donating properties than RN CD CSP, or groups that can exhibit this type of interaction may be better accessible on the former CSP. The addition of TFA to the mobile phase has an interesting effect on the values of coefficient *b*. While this is almost negligible in the system with RN CF6 CSP, the H-bond acidity increases significantly on RN CD column if TFA is present in the mobile phase. This result correlates with the retention values of binaphthyl derivatives in Table 2. Sorption of mobile phase components on the surface of a stationary phase substantially influences interaction possibilities offered by the stationary phase. TFA is a hydrogen donor and as such it can increase the *b* values if sorbed on the stationary phase. The obtained results indicate that sorption of TFA is much higher on the RN CD CSP. This corresponds to the decrease of hydrogen bond basicity (coefficient *a* values) observed on this column after addition of TFA.

The *s* regression coefficient (describing difference of polarity/polarizibility) is positive for all the studied separation systems because many polar and polarizable groups are available on the both CSPs. The value of this coefficient decreases by addition of TFA to the mobile phase for the both chiral stationary phases to a similar extent. The acid competes with the analytes for the interaction sites of this type on the stationary phases and in this way decreases their retention.

The *e* coefficient is statistically insignificant in all the chromatographic systems tested. That means that propensity of the stationary and the mobile phases to interact with solute n- and π-electron pairs is equal. It can be even further deduced that this type of interaction is related to the same substituent on CF or CD and has equal effects in all the separation systems compared in this work.

4. Conclusions

A new naphthylethyl substituted cyclofructan-based chiral stationary phase was investigated in the normal phase separation mode. Advantageous enantiodiscrimination capabilities of this CSP over a cyclodextrin based column with the same substituent (bonded to a different oligosaccharide structure) were demonstrated on a group of binaphthyl catalysts. Addition of trifluoroacetic acid to the mobile phase composed of hexane and propane-2-ol did not affect retention or enantioresolution of the analytes to a great extent with the exception of compound 5 (with ionizable functional groups) which exhibited lower retention but higher resolution of its atropoisomers in the system with TFA.

The interactions participating in the retention and enantioseparation mechanism were identified using LFER. As mentioned above the LFER model cannot reveal directly the difference between interactions of individual enantiomers that would be related to their different spatial arrangements. However, the LFER results denoted that the main impact on the interaction mechanism on the RN CF6 CSP have hydrogen bond acidity and polarity/polarizibility, while hydrogen bond basicity and interactions with n- and π-electron pairs seem to be insignificant and dispersion interactions are preffered in the mobile phase. The negligible effect of the addition of TFA to the mobile phase on the contribution of hydrogen bond acidity to the interaction mechanism also was confirmed by LFER.

Acknowledgements

We gratefully acknowledge financial support of the long-term research plan of the Ministry of Education, Youth and Sports of the Czech Republic, MSM 0021620857, of the Grant Agency of the Czech Republic, grant no. 203/08/1428, and Grant Agency of the Academy of Science of the Czech Republic, grant no. IAAX00100903.

References

[1] T.J. Ward, B.A. Baker, Anal. Chem. 80 (2008) 4363.
[2] P. Sun, C. Wang, Z.S. Breitbach, Y. Zhang, D.W. Armstrong, Anal. Chem. 81 (2009) 10215.
[3] P. Sun, D.W. Armstrong, J. Chromatogr. A 1217 (2010) 4904.
[4] C. Wang, P. Sun, D.W. Armstrong, in: A. Berthod (Ed.), Chiral Recognition in Separation Methods: Mechanisms and Applications, Springer Verlag, Berlin, 2010, p. 77.
[5] C. Jiang, M.Y. Tong, Z.S. Breitbach, Y. Zhang, D.W. Armstrong, Electrophoresis 30 (2009) 3897.
[6] M. Sawada, T. Tanaka, Y. Takai, T. Hanafusa, T. Taniguchi, M. Kawamura, T. Uchiyama, Carbohydr. Res. 217 (1991) 7.
[7] S. Immel, G.E. Schmitt, F.W. Lichtenthaler, Carbohydr. Res. 313 (1998) 91.
[8] L. Loukotková, E. Tesařová, Z. Bosáková, P. Repko, D.W. Armstrong, J. Sep. Sci. 33 (2010) 1.
[9] L. Loukotková, M. Rambousková, Z. Bosáková, E. Tesařová, Chirality 20 (2008) 900.
[10] L. Pu, Chem. Rev. 8 (1998) 2405.
[11] R. Noyori, I. Tomino, Y. Tanimoto, M. Nishizawa, J. Am. Chem. Soc. 106 (1984) 6709.
[12] P. Kočovský, Š. Vyskočil, M. Smrčina, Chem. Rev. 103 (2003) 3213.
[13] J.M. Brunel, Chem. Rev. 105 (2005) 857.
[14] Š. Vyskočil, L. Meca, I. Tišlerová, I. Císařová, M. Polášek, S.R. Harutyunyan, Y.N. Belokon, R.M.J. Stead, L. Farrugia, S.C. Lockhart, W.L. Mitchell, P. Kočovský, Chem. Eur. J. 8 (2002) 4633.
[15] M.J. Kamlet, R.M. Doherty, J.L.M. Abbound, M.H. Abraham, R.W. Taft, Chemtech 16 (1986) 566.
[16] A. Sandi, L. Szepesy, J. Chromatogr. A 818 (1998) 1.
[17] A. Sandi, M. Nagi, L. Szepesy, J. Chromatogr. A 893 (2000) 215.
[18] L. Janečková, K. Kalíková, Z. Bosáková, E. Tesařová, J. Sep. Sci. 33 (2010) 3043.
[19] M. Lämmerhofer, P. Franco, W. Lindner, J. Sep. Sci. 29 (2006) 1486.
[20] J. Jiskra, H.A. Claessens, C.A. Cramers, R. Kaliszan, J. Chromatogr. A 977 (2002) 193.
[21] S. Schefzick, M. Lammerhofer, W. Lindner, K.B. Lipkowitz, M. Jalaie, Chirality 12 (2000) 742.
[22] M.H. Abraham, A. Ibrahim, A.M. Zissimos, J. Chromatogr. A 1037 (2004) 29.
[23] M.H. Abraham, J. McGowan, Chromatographia 23 (1987) 243.
[24] M.H. Abraham, G.S. Whiting, R.M. Doherty, W. Shuely, J. Chromatogr. 587 (1991) 213.
[25] M. Vitha, P.W. Carr, J. Chromatogr. A 1126 (2006) 143.
[26] A. Berthod, C.R. Mitchell, D.W. Armstrong, J. Chromatogr. A 1166 (2007) 61.
[27] A. Nasal, P. Haber, R. Kaliszan, E. Forgacs, T. Cserhati, M.H. Abraham, Chromatographia 43 (1996) 484.
[28] M.H. Abraham, J.A. Haftvan, G.S. Whiting, A. Leo, R.S. Taft, J. Chem. Soc. Perkin Trans. 2 8 (1994) 1777.
[29] E.C. Vonk, K. Lewandowska, H.A. Claessens, R. Kaliszan, C.A. Cramers, J. Sep. Sci. 26 (2003) 777.
[30] K. Kalíková, J. Lokajová, E. Tesařová, J. Sep. Sci. 29 (2006) 1476.
[31] A.M. Stalcup, S.-C. Chang, D.W. Armstrong, J. Chromatogr. 540 (1991) 113.
[32] A. Berthod, S.-C. Chang, D.W. Armstrong, Anal. Chem. 64 (1992) 395.
[33] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73.
[34] J.L. Hintze, NCSS User's Guide II, NCSS, Kaysville, UT, USA, 2007.
[35] D.W. Armstrong, T.J. Ward, R.D. Armstrong, T.E. Beesley, Science 232 (1986) 1132.
[36] D.W. Armstrong, W. DeMond, J. Chromatogr. Sci. 22 (1984) 411.